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Mathematical modeling approaches of cellular endocrinology within the hypothalamo-pituitary-gonadal axis[☆,☆☆]

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Abstract

The reproductive neuroendocrine axis, or hypothalamo-pituitary-gonadal (HPG) axis, is a paragon of complex biological system involving numerous cell types, spread over several anatomical levels communicating through entangled endocrine feedback loops. The HPG axis exhibits remarkable dynamic behaviors on multiple time and space scales, which are an inexhaustible source of studies for mathematical and computational biology. In this review, we will describe a variety of modeling approaches of the HPG axis from a cellular endocrinology viewpoint. We will in particular investigate the questions raised by some of the most striking features of the HPG axis: (i) the pulsatile secretion of hypothalamic and pituitary hormones, and its counterpart, the cell signaling induced by frequency-encoded hormonal signals, and (ii) the dual, gametogenic and glandular function of the gonads, which relies on the tight control of the somatic cell populations ensuring the proper maturation and timely release of the germ cells.

Keywords: Hypothamo-Pituitary-Gonadal axis – mathematical models – cell population dynamics – pulsatile secretion – cell signaling

1. Biological background and review scope

The hypothalamo-pituitary gonadal axis (HPG) controls the reproductive function in vertebrates. The HPG axis ensures the proper maturation of germ cells, and the coordination of their release with appropriate internal (e.g. metabolic status) and external (e.g. daylength) environmental conditions.

As the other neuro-endocrine axes, the HPG involves three anatomic levels, the hypothalamus, the pituitary gland, and paired peripheral organs, the gonads. The reproductive function is controlled through intertwined endocrine loops connecting these levels. The hormonal dialogue is underlain by a variety of cell types, which are able to respond to, and to release hormonal signals.

On the hypothalamic level, specific endocrine neurons secrete the neuro-hormone GnRH (gonadotropin-releasing hormone) in a pulsatile manner. GnRH pulse frequency acts as a metronome, whose speed is finely tuned by gonadal signals, which are conveyed through several neural systems, with a prominent role of the KNDy (kisspeptin, neurokinin B, dynorphin) system.

On the level of the anterior pituitary, endocrine pituitary cells, the gonadotrophs, release two different gonadotropins, FSH (follicle-stimulating hormone) and LH (luteinizing hormone), in response to GnRH. Encoding of GnRH signal as pulses is preserved at the gonadotroph level, despite GnRH short half-life, thanks to the existence of a dedicated portal blood system. Changes in GnRH pulse frequency, as those engendered by the varying steroid environment along an ovarian cycle, are associated with preferential release of FSH or LH (cf Figure 1).

The gonadal level involves two types of gonadotropin-responsive cells, which share a common embryonic origin in females and males, and ultimately build up respectively the ovarian follicles or seminiferous tubules. Theca cells in the ovaries, and Leydig cells in the testes are LH-responsive steroidogenic cells. Granulosa cells in the ovaries and Sertoli cells in the testes are FSH-responsive cells supporting the maturation of the germ cells.

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They are also able to synthesize peptide hormones and participate in steroid biosynthesis in coordination with their theca/Leydig cell partners. Gonadal steroids such as testosterone, progesterone and estradiol modulate in turn the secretion of GnRH and gonadotropins, while peptide hormones such as for instance inhibin mainly act on gonadotrophs. In addition, due to the permanent remodeling of the ovaries induced by follicle development all along reproductive life, the size of the available pool of hormonally active cells is highly dynamic in females.

This review focuses on a variety of mathematical models dealing with issues of cellular endocrinology arising on the different anatomical levels of the HPG axis. We will pay most of our attention to some dynamic features specific to the HPG axis, namely: the pulse-and-surge pattern of GnRH secretion, the decoding of GnRH pulse frequency by its target cells, FSH and LH signaling in gonadal cells, and the dynamics of gonadotropin-responsive cell populations in the gonads. We refer the reader to a former review (Yvinec et al., 2018) for other interesting biomathematical issues raised by the HPG, such as (i) the detection and possible reconstruction of pulsatile secretion events, and (ii) the modeling of hormonal blood levels in males or females (ovarian cycle). We also mention recent reviews dealing with related topics: mathematical neuroendocrinology (Bertram, 2015; Leng and MacGregor, 2018), electrophysiology in pituitary cells (Fletcher et al., 2018), and general modeling issues in endocrine systems (Zavala et al., 2019).

2. From excitation to secretion: modeling approaches of GnRH neurons and gonadotrophs

2.1. Electrophysiological models for GnRH neurons and gonadotrophs

GnRH neurons and gonadotrophs share the property of being excitable endocrine cells. As such, they undergo a process of excitation-secretion coupling underlain by electrophysiological steps. There is a huge literature in electrophysiological modeling, amongst which numerous studies have been dedicated to either GnRH neurons or gonadotrophs (most of them are reviewed in (Bertram, 2015), (Fletcher et al., 2018) or (Leng and MacGregor, 2018)). We will just pick-up some instances that can give a flavor of more comprehensive descriptions.

The general framework for electrophysiological models are the famous Hodgkin-Huxley (HH) equations, coupling the change in membrane potential with the balance between inward and outward currents flowing through a variety of ion channels that are characterized by different time constants of activation/deactivation. The modeling of electrical activity can be coupled with the modeling of intracellular calcium oscillations, which are of special interest in pulsatile secretion events as GnRH and LH release. Adding diffusion terms in the HH equations can be helpful to model the dynamics of the different calcium stores. The HH equations result in multiple timescale systems that can exhibit a rich collection of behaviors such as single or repetitive spiking and different types of bursting.

As far as GnRH is concerned, the repertoire of ion channels and the calcium stores underlying excitability differ a lot according to the experimental setup (Jasoni et al., 2010), getting closer and closer to physiology from immortalized GnRH neuronal cell lines (GT1), embryonic olfactive placodes, isolated adult GnRH neurons, to perfused brain slices. Hence a model designed from and fitted to GT1 cell data cannot have the same physiological relevance as models elaborated from more realistic setups. In the same spirit, one should be very cautious when making use of a combination of data from different setups. Even in the most relevant devices, the physiological interpretation may be subject to possible methodological bias (for instance optogenetics cannot be assimilated to physiological functioning). A classical implementation of the HH formalism has been performed in (Moran et al., 2017) with the objective to recapitulate with a single model two types of bursting patterns exhibited by GnRH neurons, namely: (i) parabolic bursting, during which the spike amplitude first increases and then decreases, while the spike frequency is higher at bursting onset and offset, and (ii) irregular bursting, the most common one with no clear trend in spike amplitude or frequency. Other recent attempts in modeling GnRH excitability have intended to account for the anatomical specificities of GnRH neurons (Chen and Sneyd, 2015). Indeed, their terminations, also called "dendrons", share both axonic and dendritic properties, so that they can be the site of action potential initiation. Some of the distal action potentials may result from dendro-dendritic coupling between GnRH neurons (Campbell et al., 2009) and participate in GnRH neuron synchronization. Simulating the spatio-temporal changes in electrical and ionic activity on a realistic neuro-anatomical geometry is an interesting perspective to renew HH-based approaches in mathematical neuroendocrinology.

Since pituitary cells are excitable, yet not neuronal cells, a main question is whether they exhibit spon-

taneous electrical activity, and in how much it differs from stimulus–induced (i.e. GnRH induced) activity. Spontaneous activity in gonadotrophs is characterized by large amplitude and small duration spikes, which do not result in large calcium oscillations, hence are not sufficient to trigger exocytosis. In other words, *in vitro*, gonadotrophs do not have spontaneous secretion activity, which corresponds to a low *in vivo* basal secretion. When applying a GnRH stimulus, the Gq pathway associated with GnRH receptor is activated, which elicits bursting (Li et al., 1997). In primary cell cultures, electrical bursting is associated with large amplitude calcium oscillations (Gonzalez-Iglesias et al., 2015), which occur out-of-phase of the electrical bursts, and whose frequency are correlated with the stimulus level. In contrast, when GnRH stimulus is applied to immortalized cell lines such as αT3 or LβT2 cell lines, a spike-plateau response is observed (consisting of a calcium spike followed by decay to a plateau) as it is the case in stimulated thyrotrophs or lactotrophs. As mentioned above for GnRH, the choice of the experimental cellular setup has a great importance in interpreting the conclusions drawn by mathematical models and their physiological relevance.

The main mechanistic interest of electrophysiological models is to track or to infer the presence of a specific ion channel from electrical traces, and to proceed to *in silico* pharmacological perturbations. The ultimate step of this logic has resulted in the dynamic clamp concept, where the computer is directly related to a patch-clamp electronic device. A particularly interesting implementation of dynamic clamp has evidenced the role of BK channels (large conductance potassium) in bursting spontaneous activity (Tabak et al., 2011) as suggested by a former modeling study (Van Goor et al., 2001). Adding a fast-activating BK conductance changes gonadotroph spontaneous activity from spiking to bursting, somehow converting them in lactotroph-like cells.
Notwithstanding, one should keep in mind that there is a great between-cell heterogeneity, especially in spontaneous activity, which has to be accounted for by experimental design and associated modeling approaches.

2.2. GnRH generator

The so-called GnRH pulse generator corresponds to the neural network generating the pulsatile pattern of GnRH, including not only the GnRH neurons themselves, but also all interneurons projecting afferences to GnRH neurons. Amongst the cellular components of the controlling systems, the KNDy neuron system has taken a prominent part since its discovery through genetic studies in patients affected by central hypogonadotropism in 2001 (see the review (Pinilla et al., 2012)). For long, a set of different neurotransmitters emanating from different sites of the hypothalamus (preoptic area, arcuate and ventromedial nucleus), including for instance GABA (gamma-aminobutyric acid), endorphins, NPY (neuropeptide Y), had been thought to control jointly the GnRH system (Herbison, 1998). KNDy neurons are supposed to be part of (or even responsible for) the pulse-generating mechanism, and to process the information conveyed by gonadal steroids before forwarding them to GnRH-secreting neurons (Herbison, 2018).
Despite the involvement of more and more sophisticated experimental devices, experimental studies relating KNDy neuron activity to GnRH secretion either remain on a single cell scale (typically by detecting action potentials on GnRH neurons (Piet et al., 2015)) or use MUA (Multi-Unit Activity) or LH pulses as a surrogate for evidencing GnRH pulse events. Substituting LH to GnRH lies on the principle that GnRH pulses drive LH pulses in a one-to-one forcing, yet there do exist physiological situations of uncoupling between GnRH and LH, especially in high frequency regimes (Moenter, 2015).
Monitoring *in vivo* GnRH pulses remains extremely difficult. MUA time series have been initially used as an indirect witness of GnRH secretion (Nishihara et al., 1999). Indeed, volleys of electrical activity recorded in the medio-basal hypothalamus appeared to coincide with LH pulses detected from the general circulation.
The first modeling works on GnRH pulse generator were thus motivated by and inspired from MUA experiments. The basic ingredients of these models are stochastic point processes and their associated counting processes. They generate stochastic series of pulses, each considered as a point event occurring with an average frequency controlled by a more or less sophisticated intensity. Departure from the simplest Poisson process (with a constant intensity) can allow one to account for a time-dependent activity (and especially a circadian modulation as observed at puberty onset) and also to embed memory effects when considering the chronology of past events (Camproux et al., 1994). A step further was performed in a comprehensive work on the male HPG axis (Keenan et al., 2000), which introduced feedback terms related to testosterone concentration in the intensity formulation. In addition, pulse events are associated with varying amounts of GnRH release, resulting from the testosterone-dependent refilling rate of secretion vesicles. The whole model is for-

mulated as a system of SDE (Stochastic Differential Equations) capturing the main interactions between GnRH, LH and testoterone secretion.
In (Brown et al., 1994), the authors chose a different strategy. They used a simple Poisson process with constant intensity as an input onto an excitable dynamics, to mimic neural excitatory stimuli onto GnRH neurons. The amplitude of each stimulating event is drawn from a Gaussian distribution. Depending on the state of the excitable system, these stochastic inputs may (or not) trigger a pulse. The combination of a stochastic input and a deterministic excitable system engenders the series of simulated GnRH pulses. GnRH-induced LH release is then computed from a saturated dose-effect relation. This relation has been refined later to account for the self-priming effect of GnRH stimulation, by means of a delay differential equation (Scullion et al., 2004). From the mathematical viewpoint, an excitable system driven by stochastic inputs can be considered as an IODE (Impulse Ordinary Differential Equations) system, where stochastic inputs reset the state of a deterministic system. A completely deterministic framework encompassing a similar richness is provided by pulse-modulated systems. This framework can be used to design models of the GnRH generator as comprehensive as the the SDE-based models. At the same time, they are amenable to theoretical analysis of periodic solutions. Interestingly, such a study can exhibit regimes in which the frequency locking ratio can exceed one, hence where LH pulse frequency can be lower than GnRH pulse frequency (Churilov et al., 2009).
MUA-based modeling approaches are still being developed. In (Voliotis et al., 2019), an excitable dynamical system, exhibiting relaxation oscillations in some parameter regimes, has been designed to represent the putative pacemaker activity of KNDy neurons. The average firing rate of the system is associated with the neurosecretion of dynorphin and neurokinin B, which in turn modulates the firing activity. The firing frequency is fitted to MUA recordings, and related to LH pulses. Somehow, MUA are considered as correlates of KNDy rather than GnRH neuron activity, under the assumptions that KNDy pulses drive GnRH pulses, which are themselves witnessed by LH pulses.
After MUA, a new generation of experimental measurements, much more reliable, was made available, which has still not been overpassed by any other approach. It consists in high rate sampling of hypophyseal portal blood flow *in vivo*, giving access to direct information on GnRH pulses. Moreover, such a device has allowed endocrinologists to investigate in depth the control exerted by gonadal steroids in castrated animals. In ovariectomized ewes, exogenous administration of estradiol and progesterone gave a very accurate insight into their respective effect, not only on GnRH pulse and the change in GnRH frequency along the ovarian cycle, but also on the transition from the pulsatile regime to a surge regime in the preovulatory period (Christian and Moenter, 2010). Simultaneously, the challenge got higher for GnRH modeling since it requires capturing both the coordinated changes in frequency and back and forth transition from the pulse to the surge regime.
A series of modeling works was dedicated to designing and analyzing a model meeting all qualitative and quantitative specifications imposed by the neuroendocrinology of the ovarian cycle. The GnRH pulse and surge generator model was introduced in (Clément and Françoise, 2007), deeply analyzed mathematically in (Clément and Vidal, 2009), and thoroughly interpreted from the endocrinological viewpoint in (Vidal and Clément, 2010; Clément and Vidal, 2016). The model consists of a secreting system controlled by a regulating system. It is a compact (4D), highly nonlinear, slow-fast dynamical system with 3 timescales, which reproduces the basic pulsatile behavior, the increase in frequency observed between the luteal and follicular phase, the transition to surge and pulsatility resumption (Clément and Françoise, 2007). The hysteresis dynamics of the regulating system embeds the steroid feedback exerted onto the GnRH neurosecretory system, and especially the surge-triggering effect of estradiol and pulse-modulating effect of progesterone (see (Herbison, 2020; Moenter et al., 2020) for a comprehensive review of steroid control of the pulse and surge generator). The parameters can be calibrated to fit the proper durations of the different phases (luteal, follicular, surge) and the relative change in portal GnRH levels in different species (Clément and Vidal, 2009). *In silico* experiments can also reproduce the effect of a bolus administration of estradiol or progesterone on GnRH pulse frequency, as well as the progesterone-induced blockade of GnRH surge triggering (see (Vidal and Clément, 2010) and references therein to the corresponding experimental studies). Recently, a 6D version with two secretor systems has been proposed to represent the desynchronization observed in GnRH secretion just before the surge (Köksal Ersöz et al., 2018).
Even if pulse generation might have an external origin rather than being underlain by intrinsic properties of GnRH neurons (Herbison, 2018), the fact remains that the genesis of GnRH pulses on the proper endocrine timescale is a network emergent behavior.
Seminal studies performed on olfactory placodes (where GnRH neurons originate from during de-

velopment) have clearly illustrated that synchronized GnRH secretion events are associated with synchronized calcium oscillations organizing from background desynchronized, yet oscillatory calcium dynamics in individual neurons (Terasawa et al., 1999).

This synchronization behavior was captured both qualitatively and quantitatively by a network model including several tens of GnRH neurons (Krupa et al., 2013). The model outputs give the simultaneous changes in intracellular calcium concentration in each neuron. Individual dynamics are ruled by a 3D slow-fast dynamical system coupling the calcium with electrophysiological-like variables (a firing and a recovery variable). Each neuron exhibits oscillatory calcium dynamics whose period and amplitude are distributed within a range compatible with experimental data (typical interpeak intervals of 6-8 min in rodents). When the individual dynamics are coupled through a volume transmission mechanism (global coupling), synchronized calcium peaks occur on a much slower time scale (50-60 min). The dynamical mechanism underlying synchronization involves complex oscillations (mixed-mode oscillations). The model also accounts for the silent period observed after synchronization events, before individual oscillations resume again. In addition, it can reproduce other, less frequent experimental observations, such as synchronized pulses resulting from the partial recruitment of neurons (versus all neurons) or the occurrence of doublets of synchronization. The key point is that, in contrast to apparent synchronization produced by the superimposition of individual peaks occurring at the same time, here a true synchronization process emerges from the interaction between neurons.

Going further into the modeling of the GnRH generator as a network of coupled neurons faces a not yet surmounted difficulty. Very little is known about the network topology and connectivity between the involved neurons, whether they be the endocrine neurons themselves (Constantin, 2017) or the regulating neurons such as KNDy neurons. Quantitative information are sorely lacking; what is the size of these networks and how many neurons are involved in the generation of pulses or surge? Interestingly, the regulating neurons integrating the steroid signal that triggers the surge are located in different neuroanatomical sites than the neurons integrating the steroid signal modulating pulse frequency, without precluding interconnectivity (Herbison, 2020; Moenter et al., 2020). They also appear to convey these signals in a different manner, by targeting preferentially GnRH neuron terminations to control pulse release, and cell bodies to control surge triggering. As stated in (Moenter et al., 2020) "How these signals are generated in these cells and then conveyed to GnRH neurons largely remains a mystery. Mechanistic studies of population synchrony and the neurobiology of the interactions between kisspeptin neurons and GnRH neurons need to be pursued".

2.3. GnRH signaling in gonadotrophs

As objectivized by direct measurements in the pituitary portal blood, GnRH pulses are shaped as a square-wave signal (Moenter et al., 1992), characterized by its frequency (inverse of the inter-pulse interval - IPP), amplitude and duration, and a long (baseline) off-state with respect to the on-state, leading to a short duty cycle.

GnRH[1] targets its cognate GPCR (G protein-coupled receptor), GnRHR, and triggers a signaling cascade controlling eventually the secretion of both FSH and LH (for a review, see for instance (Bliss et al., 2010; Naor, 2009)). High frequency pulses, as encountered during the end of the follicular phase of the ovarian cycle, favor LH secretion, while lower frequency pulses favor FSH secretion.

On the molecular level, the differential control of GnRH has been best evidenced on transcription, hence the first step in the whole secretion process (reviewed in (Stamatiades and Kaiser, 2018)). Studies performed *in vitro*, either in primary monolayer cultures of rat pituitary cells (Kaiser et al., 1997) or perifused LβT2 cell line, a murine gonadotroph-derived cell line (Bedecarrats and Kaiser, 2003), and *in vivo* on castrated, testosterone-replaced rat males (Dalkin et al., 1989), have shown that the expression of both βFSH and βLH subunits vary according to a bell-shaped curve as a function of (physiologically-relevant) GnRH pulse frequency, with a lower optimum frequency for FSH than LH. The *in vitro* studies have also suggested that there are concomitant changes in the cell-surface GnRHR density, which is reminiscent of the priming effect of GnRH on its own signaling, occurring in particular at puberty and during the preovulatory period.

Importantly, in both *in vitro* and *in vivo* setups, such effects were still observed when the total GnRH dose administered was kept constant, suggesting a possible "true" frequency effect in contrast to a cumulative dose effect. Its is worth noting that compensation for frequency increase through alteration in the pulse amplitude and/or duration is likely to occur in physiological situations. More precisely, the estradiol-induced frequency increase

[1] Anywhere in this article, we use GnRH to designate GnRH I, and GnRHR, the mammalian GnRH receptor type I, lacking a carboxyl-terminal tail (see (Millar et al., 2004) for a review on GnRH variants and GnRHR types).

preceding the ovulatory surge seems to be accompanied by a change in the shape of the pulse (that becomes sharper and lower) and by no increase, and even a decrease in the cumulative dose (Evans et al., 1994). However, discriminating effects induced by cumulative dose or frequency remains an unreached experimental challenge, partly because the observed response (mRNA in this context) is obtained as endpoint observations, while much more time-resolved data would be needed.

Many mathematical models have dealt with the control of gonadotrophin secretion by GnRH (cf Figure 2).

A first wave of works focused on the GnRH-induced LH release through calcium-dependent exocytosis of secretion granules, and were motivated by experiments performed on perifused (rat or ovine) pituitary cells. In (Blum et al., 2000), the authors investigated the effect of extracellular calcium on LH release in response to single GnRH pulses. Their ODE (ordinary differential equations)-based model consists of a proximal signaling module – describing receptor binding and activation of Gq protein leading to IP3 (inositol trisphosphate) production – and a simplified, non-oscillating calcium signaling module – ruling the average cytosolic calcium level from inward and outward fluxes from the reticulum and extracellular medium –. LH release is then simply a (second-order) Hill function of cytosolic calcium. The model in (Heinze et al., 1998) is even simpler since the LH release is directly determined by the level of GnRH in the ligand-bound state, while a desensitized state is introduced to try to explain the apparent attenuation in the LH response after exposure of ovine cells to consecutive GnRH pulses. In contrast, (Evans et al., 2013) intended to reproduce an amplification in the LH response in a similar culture setup, yet using rat pituitary cells. The apparent contradiction might be explained by interspecific differences in the molecular structure of GnRHR in sheep compared to rat, associated with an increased internalization rate (Arora et al., 1999). The signaling components of the model are even more simplified, and amplification results essentially from a progressive increase in baseline levels.

A second wave of works was explicitly motivated by GnRH-induced transcription, and especially the frequency-dependent control of gonadotropin subunit (GSU) transcription. Most of them include a similar proximal G protein-dependent signaling module as that introduced in (Blum et al., 2000) (and slightly completed in (Washington et al., 2004)) and consider in addition one or several mitogen-activated protein kinase (MAPK) cascades. In its most complete formulation, the model introduced in (Lim et al., 2009) provides a comprehensive description with up to four MAPK pathways (ERK1/2, JNK, p38, and ERK5) in parallel interacting through DUSP (dual specificity phosphatase) mediated crosstalks. The main outputs of the model, the respective transcription rates of α, βFSH and βLH GSU, are computed from a combination of these MAPK pathways. The model introduced in (Tsaneva-Atanasova et al., 2011) also considers interaction/cooperation between different transcription factors as the source of differential control of GSU transcription. In contrast to (Lim et al., 2009), a single MAPK-like cascade (ERK, extracellular signal regulated kinase) is considered, yet it is combined with NFAT (nuclear factor of activated T-cells)-induced transcription. Since activation of NFAT is a calcium-dependent process, requiring the binding of calcium ions to calmodulin, the model also embeds a simplified calcium signaling module as introduced in (Blum et al., 2000). The results suggest that cooperation between NFAT and ERK is needed to observe frequency dependent effects. The difference of viewpoints between these two approaches may be associated with their making use of different experimental data. (Lim et al., 2009) refers to data retrieved in the LβT2 and αT3 cell lines, which are both immortalized gonadotroph cell lines, hence expressing GnRHR constitutively, while (Tsaneva-Atanasova et al., 2011) refers to data retrieved in the HeLa cell line, which derives from human cervical cancer and in which GnRHR is over-expressed. As mentioned in section 2.2, the issue of which experimental device (type of immortalized cell lines, primary cultures, *in vivo* or *ex vivo* studies ...) a mathematical model is paired with, is a common barrier to compare the assumptions and results of different models dealing with a similar biological question.

Although they complete the picture of GnRH signaling pathways in gonadotrophs, the main caveat of these works is that they study the cumulative effect associated with increased pulse frequency, and not a true frequency effect, since simulations are performed without compensating for the cumulative dose.

The latest wave of works addressed frontally the question of a true frequency effect. In (Fletcher et al., 2014), the authors have introduced the appropriate theoretical framework to investigate the information encoded by frequency in square wave-signals. They have discriminated compensating for frequency increase by reducing either duration or amplitude, proposed to use as proper output the average response value over an oscillation period (over the time interval between two consecutive pulses), once the oscillatory steady state regime due to periodic forcing has been reached. They

have also exhibited behavior rules allowing or not for a true frequency effect in simple biochemical motifs. The study in (Stern et al., 2017) deployed these concepts in a thorough manner and specifically on FSHβ GSU transcription. The authors compared, according to their frequency-detection properties, three different compact modules for either direct or indirect activation of FSHβ GSU transcription subject to concomitant inhibition, each of them compatible with the bell-shaped pattern. They illustrated their results by means of useful color-maps, in which level sets corresponding to a conserved cumulative dose can be followed along frequency changes. Although they significantly enriched the available data on FSHβ GSU transcription in LβT2 cells, by performing high-throughput mRNA detection, the authors conclude that it remains difficult to distinguish between a true frequency effect and a cumulative dose effect, and that both can coexist.

In addition, subject to including in the models appropriate ingredients, part of the distinction ensues rather from a quantitative than qualitative viewpoint, and relies on properly tuning the parameters values of the saturating functions in the upstream modules (which can filter out amplitude effect as can do rapid desensitization), and the time constants in the downstream modules (which can maintain signaling effects during the off-period). Beyond this, dissecting, in either case, what in the cell response is due to the amplitude, duration and period of the GnRH pulses remains a challenging question. Some steps forward in this direction have been made in a theoretical biology study (Krakauer et al., 2002), which has characterized the response to duration and frequency (keeping amplitude constant) in formal signaling modules possibly involved in GnRH signaling pathway (feedforward activation in series or parallel, and activation subject to feedback).

These principles are operating in the other modeling studies that have paralleled the true frequency effect and cumulative dose effect, yet in a less systematic way. The study in (Magill et al., 2013) focused on comparing the effects of two frequency regimes (30 or 100 min IPP) and tuned the parameters accordingly, even if the results can possibly be extrapolated to a larger range of frequencies. As in (Stern et al., 2017), the model plays with the antagonism between stimulating (such as CREB, cAMP response element-binding protein) or inhibiting (such as ICER, inducible cAMP early repressor) transcription factors induced concomitantly by GnRH signaling, and use compact signaling modules, except a more detailed distal signaling module dedicated to nuclear steps leading to transcription.

Finally, an original approach tackled the question from a signal engineering viewpoint, applying the concepts of information transfer (Voliotis et al., 2018). The main interest is to take advantage of the information processing properties resulting from the heterogeneity of responses within a cell population. A compact statistical index, the mutual information (MI) can be computed from single cell experimental data or from synthetic data. In the latter case, individual cell responses are simulated from a classical ODE-based model (slightly modified from (Pratap et al., 2017), itself derived from the initial ERK/NFAT model (Tsaneva-Atanasova et al., 2011)) including stochastic components, such as time-fluctuating concentrations of GnRHR or calmodulin. Comparing the indexes obtained with different parameter configurations can highlight the most efficient way to transfer information reliably. For instance, altering the feedback rate within a signaling sequence leading to ERK phosphorylation suggests that intermediate levels of feedback are the best.

All these latest approaches are still dependent upon the experimental device, and they give some insight into the frequency control of GSU, yet each model only covers a part of the question. Indeed, GnRH signaling in gonadotrophs also involves coordination between the synthesis and secretion steps, and is subject to endocrine and paracrine modulators, amongst which the activin-follistatin-inhibin system, whose role has been known for long, and the more recently discovered role of AMH (anti-Müllerian hormone) (Garrel et al., 2016).

3. Gametogenetic and hormonal functions of the gonads: modeling approaches of somatic gonadal cells

3.1. Signaling and decision making in gonadal cells

The endocrine and exocrine functions of the gonads are supported by assemblies of somatic cells, some of them interacting directly with the germ cells. In the testes, Sertoli cells nurse the male germ cells throughout the spermatogenic process, up to the release of spermatids into the epididymis. In the ovaries, granulosa cells shelter the oocytes and ensure their maturation up to ovulation. Both cell types are able to secrete a variety of peptide or steroid hormones, as a function of their differentiation degree, and are associated with other steroidogenic cells to build-up well-defined anatomical structures: ovarian follicles (oocyte, granulosa and theca cells) and seminiferous tubules (spermatids, Sertoli and Leydig cells). The gametogenic potential is subject, both quantitatively and qualitatively,

to the proper differentiation of Sertoli cells (before puberty) or granulosa cells (during the development of ovarian follicles), and tightly controlled by the pituitary gland, according to the "two-cells/two-gonadotropins" principle. Sertoli and granulosa cells are endowed with FSHR, while Leydig and theca cells are endowed with LHR, so that the different steroidogenesis steps are perfomed in a complementary and coordinated manner in the different cell types.

Gonadotropin signaling Although FSHR and LHR are GPCRs as GnRHR, they have given rise to much less modeling works. Yet, FSH and LH signaling also raises challenging modeling questions, among which the long term changes induced in the phenotype of their target cells are of crucial physiological importance. In both Sertoli and granulosa cells, FSH signaling progressively drives the cell status from an immature, proliferating cell, to a mature, highly specialized cell (Gallay et al., 2014; Clément et al., 2020). Such a switch occurs in a rather synchronous manner in Sertoli cells at the puberty transition (cf Figure 3), while it spreads all over reproductive life in females as a consequence of the asynchronous development of ovarian follicles (cf section 3.2). Understanding this proliferation-to-differentiation switch from the signaling viewpoint requires to manage not only to characterize the differences in the signaling network topology and reaction kinetics rates associated with a given cell status, but also to decipher the slow dynamics (compared to the signaling timescale of minutes to hours) underlying cell decision making.

In Sertoli cells, the switch is associated with a reversal in the balance between the main second messengers, cAMP and PIP3. The efficiency of FSH-induced cAMP production rises from birth to puberty (Crépieux et al., 2001), whereas the sensitivity to FSH of PIP3 production decreases over time (Musnier et al., 2009). PIP3-dependent molecular events (cf Figure 3), such as mTOR and p70S6K phosphorylation, appear to be involved in the mitogenic effects of FSH, that are in turn counteracted by AMPK and PTEN (Dupont et al., 2010; Riera et al., 2012). The developmental regulation of p70S6K phosphorylation has been formalized as an ODE-based model (Musnier et al., 2009), that simulates developmental variations in the synthesis of cAMP and PIP3 and their outcome on the mTOR pathway.

Similarly, in granulosa cells, the switch is clearly associated with an increased efficiency in FSH-induced cAMP release (Henderson et al., 1987), consistently with a possible activation of cyclic kinase inhibitors by supra-threshold cAMP levels (Graña and Reddy, 1995), and occurs without significant changes in the number of FSH binding sites available per cell (Abdennebi et al., 1999; Camp et al., 1993). A compact ODE model reproducing the long term cAMP response in granulosa cells (Clément et al., 2001) suggests that auto-amplification (hence frankly nonlinear dynamics) at the cAMP synthesis step (activation of adenylyl cyclase) could be a key process. However, the biochemical bases of this amplification remain poorly understood.

Further mechanistic insight could be obtained from studies on heterologous cell lines. Indeed, a major conundrum in using immortalized Sertoli or granulosa cell lines is that expression of FSHR progressively vanishes, in relation to FSHR pro-apoptotic effects *in vitro*. Alternatively, FSHR can be introduced in non-gonadal immortalized cells, such as HEK293 cells (Human Embryonic Kidney), provided that the number of receptors at the cell surface is limited to physiological amounts, otherwise the dynamics of downstream signaling pathways may be altered. Heterologous cell models are convenient for constraining and validating dynamic models, because real-time data such as resonance energy transfer experiments (Ayoub et al., 2015) are gathered more easily than in natural gonadal cells. Still, one has to be aware of their limits, and check the existence of putative mechanisms in native cells.

Heterologous cell models have been instrumental in gaining insight into the kinetics of ERK activation by GPCRs. A seminal instance is the involvement of scaffolding proteins coupled to the internalization machinery (clathrin-coated pits), the β-arrestins, in GPCR signaling, while they had been known for long only for their role in receptor desensitization (Reiter and Lefkowitz, 2006). β-arrestins support sustained signaling via the MAPK pathway compared to the transient signaling induced by the canonical G-proteins. A thorough modeling work (Heitzler et al., 2012) has implemented this renewed paradigm to account for the kinetic pattern of ERK activation, and confirmed the prominent role of GRKs (G protein-coupled Receptor Kinases) in targeting receptors to β-arrestin mediated desensitization or signaling. Even if this approach was applied to the angiotensin (AT1AR) receptor in HEK293 cells, the results are conceptually relevant for FSHR and LHR.

One key point in testing mechanistic hypotheses is the availability of pharmacological tools allowing one to selectively inhibit or activate specific intracellular pathways, such as, respectively, silencing RNA and biased ligands. From the statistical viewpoint, reliable bias quantification is a tricky issue: one has to recover from a given set

of experimental readouts (e.g. ERK activation), representing only a small part of the whole network nodes, the ligand-specific kinetic rates, and to detect possibly significant statistical differences (Landomiel et al., 2019).
Currently, another emerging paradigm is the critical importance of compartimentalization and trafficking in GPCR signaling (Vilardaga et al., 2014). In the case of gonadotropin receptors, very early endosomes seem to have a prominent role and act as crossroads in intracellular receptor rerouting (Sayers and Hanyaloglu, 2018). Such a paradigm opens new perspective for modeling approaches. The next model generation will have to cope with the segregation of biochemical species within the cell compartments, as well as with the passive or active exchanges between compartments, for instance using a PDE framework with transport and diffusion terms.
Put together, all mechanistic information can lead to a complete panorama of the gonadotropin signaling network (Gloaguen et al., 2011; Telikicherla et al., 2011; Ulloa-Aguirre et al., 2018), which can in turn guide modeling studies or more physiologically-oriented investigations.

Steroidogenesis Mature steroidogenesis is a hallmark of differentiated Sertoli and granulosa cells. The interest in the modeling of steroidogenesis pathways has been renewed in the context of endocrine disruptors (ED). An early work (Becker et al., 1980) had focused on weighting quantitatively the different enzymatic steps possibly participating in the conversion of progestagens (progesterone or pregnenolone) into androgens (androstenediol or testosterone) in a system of perifused testis explants from rats and rabbits. A simple reasoning, based on steady-state and homogeneization assumptions, was used to assess the relative contribution of a specific enzymatic reaction as the ratio of the secretion rate of the reaction product to the sum of the secretion rates of all involved reactants (substrates and products). In a relatively similar spirit, another, more recent work (Quignot and Bois, 2013) has assessed enzymatic fluxes in the aromatization chain converting androgens to estrogens, in which the final ratio between estrone and estradiol is subject to the relative activity of two steroidogenic enzymes (17βHSD type I or II), whose expression (mRNA transcripts) and bioactivity were measured in rat granulosa cells. This biochemical process is particularly relevant since many ED perform as aromatase inhibitors or activators. The flux analysis uses a dynamic, ODE-based model. It accounts for the partition coefficients of the molecules in the different compartments (granulosa cells versus culture medium *in vitro* / granulosa cells versus other cell types and extracellular space *in vivo*) and includes nonlinear competition terms in the case of substrates metabolized by a same enzyme. Some fish species are more and more used in toxicological studies. In the fathead minnow, the whole sequence of steroidogenesis, starting from cholesterol uptake, has been represented through linear ODEs with first-order transport and synthesis/degradation terms (Breen et al., 2007). This simple model can be looked at steady state, to identify the most important steps in steroid outputs. It can also be embedded in more comprehensive setups, in particular within so-called qAOP (quantitative adverse outcome pathway) frameworks, which intend to link the exposure to ED to their adverse biological outcomes (Conolly et al., 2017).

3.2. Pool of hormonally active cells in the gonads

The size of both the Sertoli and granulosa cell pools are controlled by gonadotropins, and especially FSH. As detailed in section 3.1, the control is exerted through a switch from proliferation to differentiation. This switch occurs around puberty in males, and on the whole population level, so that the number of Sertoli cells is settled once for all. In females, the ovarian function is distributed over numerous ovarian follicles, which activate asynchronously, develop slowly and possibly degenerate at any developmental stage. As a result, even in adults, the endocrine status of the ovaries is changing as a function of the total number of granulosa cells and their distribution into different maturity levels. On the short term, i.e. on the horizon of an ovarian cycle (several days to several weeks depending on the species), there are periodic fluctuations in the number of cells participating in the endocrine loops within the HPG. The cyclic recruitment of a follicle cohort is accompanied by an increase in the ovarian output of inhibin first, and then estradiol, whose level dramatically increases in the preovulatory period. The estradiol-dominated follicular phase is followed after ovulation and corpus luteum (CL) formation by the progesterone-dominated luteal phase. On the longer term, i.e. on the horizon of the reproductive lifespan, the progressive exhaustion of the pool of quiescent primordial follicles also impacts the endocrine dialogue within the HPG. During ovarian aging, while activation of remaining follicles is enhanced due to the decrease in early developing follicles, which are responsible for most secretion of AMH, a major modulator of activation, the recruitment is accelerated and the selection of ovulatory follicles becomes less stringent. In humans for instance, theses changes result in a

shorter follicular phase (hence shorter ovarian cycle) in the perimenopausal period and increase in the occurrence of multiple ovulations (Broekmans et al., 2009).

We are not aware of any modeling work dedicated to the pool of Sertoli cells. In contrast, the dynamics of granulosa cells in ovarian follicles has been the matter of a series of works dealing with activation (Clément et al., 2019), early development and terminal development (reviewed in (Clément and Monniaux, 2013; Monniaux et al., 2016)). On a cell biology ground, these models monitor events such as transformation of one cell type into another (as the flattened to cuboidal transition encountered at activation), proliferation, terminal differentiation and apoptosis.

They are formulated within the framework of structured population dynamics, more specifically non conservative transport equations in the case of deterministic models, and measure-valued stochastic processes in the case of stochastic models. Such a formalism allows one to follow the total number of cells, as well as their distribution according to so-called structuring variables, that position cells within a physical or functional domain.

In the early stages, the control of cell population dynamics is mainly exerted through the paracrine dialogue coupling the oocyte growth with granulosa cell proliferation (Clément et al., 2013b; Clément et al., 2019). Hence, the germ cell is an indirect player in the HPG endocrine game, since it largely contributes to the proliferation of cells that will ultimately participate in the endocrine function of the ovaries.

From the HPG viewpoint, the selection of the ovulatory follicle(s) is one of – if not the – most integrative process (Clément, 2016): (i) it is the endpoint of the several-month process of follicle morphogenesis, (ii) it involves a tightly coordinated endocrine sequence : drop in FSH due to the inhibin and estradiol mediated feedback onto the pituitary gland, increase in GnRH frequency and triggering of the GnRH surge due to estradiol feedback onto the hypothalamus and estradiol priming on the pituitary level.

A multiscale model embeds most of these processes in a unified framework accounting for both the cell dynamics of individual follicles, and the coupling between the trajectories of growing follicles ensuing from the hormonal control of selection (Echenim et al., 2005; Aymard et al., 2016). The granulosa cells are characterized by their proliferative status and maturity. The progression of cells along the cell cycle and the maturation speed are tuned by a control variable representing the bioavailable FSH. The maturity variable represents the ability of cells to secrete ovarian hormones, hence to participate in the endocrine game. In the same way, the contribution of a follicle to the ovarian feedback is expressed as a weighted sum over all its cells. The model outputs combine endocrine information (decrease in FSH, timing of the ovulatory surge) with multiscale cell biology information: cell number in each follicle, growth fraction (proportion of proliferating cells), distribution of proliferating cells within the cell cycle phases (cf Figure 4). Such a model helps us to think about the co-evolution between the sensitivity of the pituitary and hypothalamus to ovarian hormones on one side, and the response of follicular cells to gonadotropins on the other side. For instance, the management of the follicle proliferative resources must be finely balanced, and there exists an optimal compromise between a safer, cautious strategy, on one hand, that would produce cells in excess, ensuring robustness to apoptosis at the expense of being "too late" to participate in the endocrine dialogue, and an eager, consuming strategy that would switch to terminal differentiation too early and deprive the follicle from reaching the critical cell number compatible with ovulation (Clément et al., 2013a).

In the case of selected follicles, granulosa cells become endowed with LHR, in addition to FSHR, while they progressively lose their ability to respond to FSH. After ovulation, theca and granulosa cells transform respectively into the LH responsive small and large cells of the corpus luteum (CL), which also contains endothelial and stroma cells. These cellular components have been considered to model the growth of the CL (Prokopiou et al., 2013). The dynamics of the different cell populations, and more specifically the volume occupied by each cell type, are ruled by ODE-based growth models, accounting for the averaged proliferation rate – for endothelial cells, to mimic angiogenesis –, or swelling rate (for all other cells) and saturation due to competition for space. The critical assumption of this model is that growth is inhibited beyond a threshold CL volume, that should not exceed that of the ovulated follicle. This assumption results in a hybrid, piecewise smooth formulation of the model, whose behavior can be thoroughly studied using Filippov theory for sliding modes.

There has been an early interest for the modeling of the long term evolution of the follicle population (Faddy et al., 1976), which has been renewed recently thanks to the discovery of AMH as an endocrine marker of the ovarian reserve. AMH blood level is directly related to the number of small growing follicles and is largely correlated to the so-called antral follicle count (AFC), which requires invasive ultrasound examination. AMH levels decline with aging, and several studies have

attempted to match AMH levels at a given age with the age at menopause (van Disseldorp et al., 2008). Most of these models are statistical regression models using linear or polynomial models to predict AMH level (Kelsey et al., 2011), or the number of non-growing follicles, as a function of age (see (Wallace and Kelsey, 2010) and references therein, and (Coxworth and Hawkes, 2010) for a statistical comparison between the main types of regression models). Other approaches embrace the whole follicle population, or focus on the quiescent pool. They are based on deterministic, or more rarely stochastic compartment models (cf Figure 5) and infer the growth and death rate within each compartment from experimental follicle counts (Faddy et al., 1976; Faddy and Gosden, 1995). These rates are supposed to be constant or at best piecewise constant with age. Despite their obvious interest, all these models are not mechanistic and the endocrine (or paracrine) landscape is missing.

A step forward in that direction has been performed in (Margolskee and Selgrade, 2013). The authors have extended their substantive modeling framework for the ovarian cycle (see (Yvinec et al., 2018) for a review on ovarian cycle modeling) to account for long term effects of the exhaustion of early growing follicles. The extended model can reproduce the continual drop in AMH, the decrease in inhibin B at mid-reproductive age, and the subsequent rise in FSH. In a similar way as (Prokopiou et al., 2013), phenomenological growth models are used to represent the different follicle classes, and the equations rule the dynamics of the volume occupied by the follicles rather than follicle numbers, except for the earliest primordial and primary stages.

Recently, a new multiscale model of the whole process of follicle development on a lifespan horizon has been introduced in (Bonnet et al., 2020). This model is implemented in different formulations, all entering the framework of structured population dynamics: as a nonlinear compartmental ODE model, as a nonlocal and nonlinear PDE model based on transport equations, and as a stochastic Continuous time Markov Chain model with nonlinear intensities. In any case, the follicle population is structured according to a maturity variable, such as follicle size. The model monitors both the progressive exhaustion of the quiescent pool, and the distribution of the growing follicles into the different maturity stages. Thanks to the differences of timescales between the activation process, early growth and terminal development, the model can be reduced and studied by means of timescale separation techniques. This model is particularly suited for studying how the follicle distribution is shaped by both the asynchronous activation of quiescent follicles and the multiple interactions between follicles resulting from paracrine and endocrine signaling. In particular, one can investigate the auto-similarity of this distribution (conserved shape despite decreased amplitude with age), and track the mechanistic links between the quiescent pool ("static ovarian reserve") and the pool of small antral follicles ("dynamic reserve") which are the target of ovarian stimulation treatments (Monniaux et al., 2014).

4. Conclusion

In this review, we have presented a variety of modeling approaches dealing, separately or jointly, with the double, endocrine and gametogenic, function of the HPG axis. To avoid redundancy with other reviews dedicated to related topics, we have focused on two particularly integrative processes, the coupling between excitation and secretion, and the control of gonadal cell pools. The coupling between very fast excitation on the single cell scale and secretion rhythms on the endocrine system level is a property shared with other neuroendocrine axes. Yet, the HPG axis is higly specific, with a single neurohormone GnRH inducing the synthesis of two different pituitary hormones, FSH and LH, from a single cell type. FSH and LH themselves target two different gonadal cell types, which in turn secrete several steroid or peptide hormones, each exerting differential yet coordinated feedback effects on the hypothalamus and/or pituitary gland. Another striking feature of the HPG axis lies in the indirect involvement of germ cells in the endocrine exchanges between the gonads and central components. In females, even in adult organisms, the hormonal output of the ovaries is underlain by developmental processes supporting the morphogenesis, growth and maturation of ovarian follicles. Such specific features raise challenging questions on the encoding and decoding of hormonal signals, not only on the individual cell level, but also on the cell population level. Some of these questions, including the alternating pulse and surge pattern of GnRH, the frequency modulated release of gonadotropins, FSH and LH signaling in the gonads, and the tightly controlled cell dynamics in ovarian follicles have been partially deciphered from both the experimental and modeling viewpoint. Yet many pieces are missing to draw the whole picture, and especially to understand the mechanistic link between dynamics observed/modelled on different scales.

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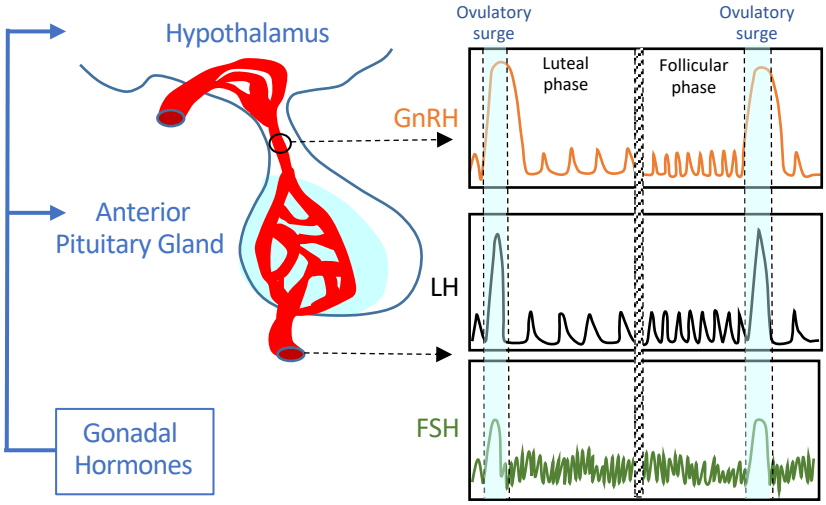


Figure 1: Encoding of GnRH, LH and FSH signals

The neurohormone GnRH is secreted as square-wave like pulses from the terminations of hypothalamic GnRH neurons into the pituitary portal vessels. In response to GnRH, the gonadotrophs secrete the gonadotropins FSH and LH. The difference in the secretion pattern of FSH compared to LH results from differences in the secretion modes and a much longer half-life. Also, the synthesis of the specific subunits βFSH and βLH is differentially controlled by GnRH pulse frequency, with a higher frequency favoring LH versus FSH.
The box inserts illustrate the changes in the secretion patterns of GnRH, LH and FSH along an ovarian cycle. The schematic secretion patterns are drawn from high resolution measurements of GnRH in the pituitary portal blood in both the pulsatile and surge regimes (Evans et al., 1994, 1995; Moenter et al., 1990) and direct cavernous sinus sampling for LH and FSH (Clarke et al., 2002).
In males, the GnRH-LH-Testosterone axis exhibits oscillations with a quite stable frequency (for a given physiological status). In contrast, in females, there are sharp contrasts in GnRH frequency along the ovarian cycle. GnRH pulse frequency is lowest during the progesterone-dominated luteal phase, while it increases during the estradiol-dominated follicular phase, and gets higher and higher until the GnRH surge occurs. The rope-like vertical bar delimits the transition between the luteal and follicular phase. The total number of pulses in the course of a cycle and each phase depends on the species. For sake of clarity, only a few pulses are represented in each phase, and the pulse amplitude has been exaggerated with respect to the surge amplitude. See the model outputs in (Clément and Vidal, 2009) for instances of secretion patterns meeting specific quantitative specifications in sheep and monkey. The duration of the GnRH surge largely exceeds that of the LH surge (Caraty and Skinner, 1999).

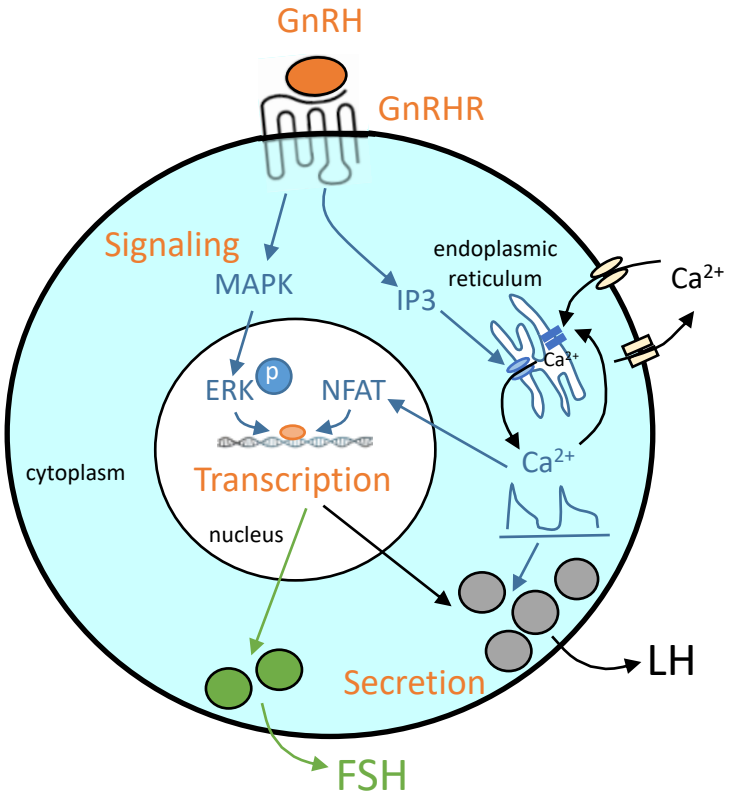
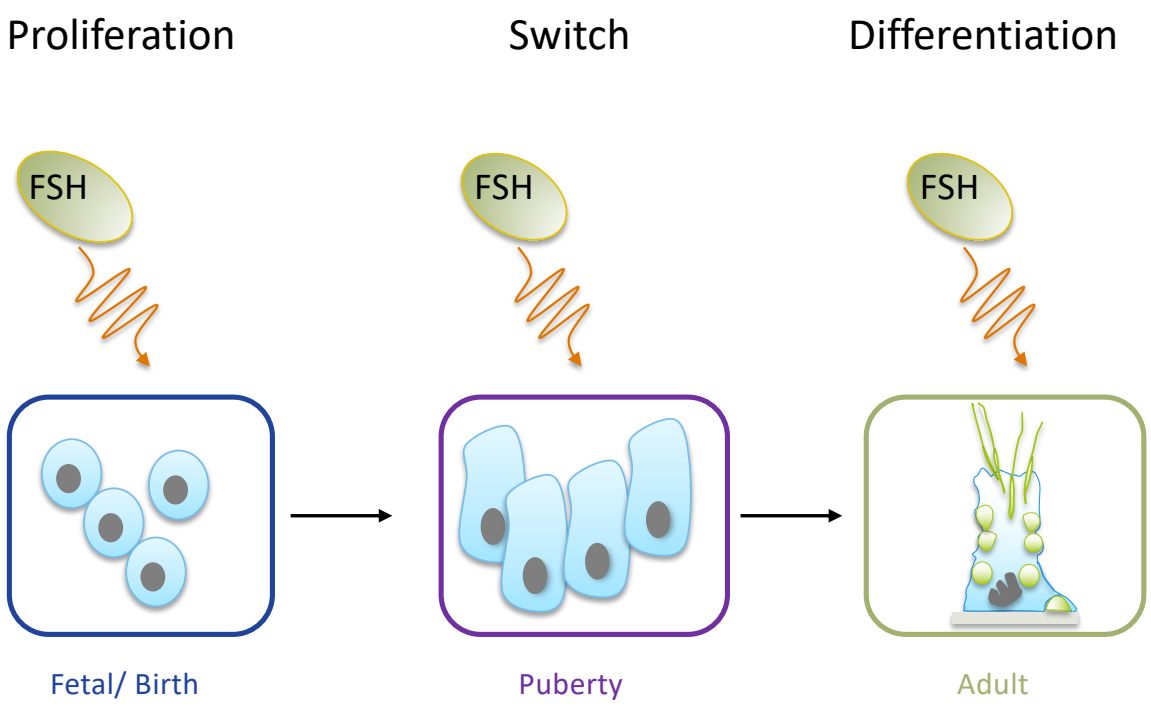


Figure 2: Decoding of GnRH in gonadotrophs

GnRH binding to its cognate GPCR receptor triggers biochemical signaling pathways, which are involved in the control of both the synthesis and release of FSH and LH. The control of the transcription rate of the α, βFSH and βLH subunits appears to be mediated by both the MAPK pathway –through co-activator as pERK– and calcium signaling –through activation of transcription factors as NFAT by a calmodulin-activated kinase–. The control of LH release from exocytosis granules is driven by oscillations in intracytoplasmic calcium, generated from the uptake and release of calcium from the endoplasmic reticulum, and calcium inflow/exflow through ion channels on the membrane. In contrast, FSH is rather secreted in a constitutive manner (Durán-Pastén and Fiordelisio, 2013).



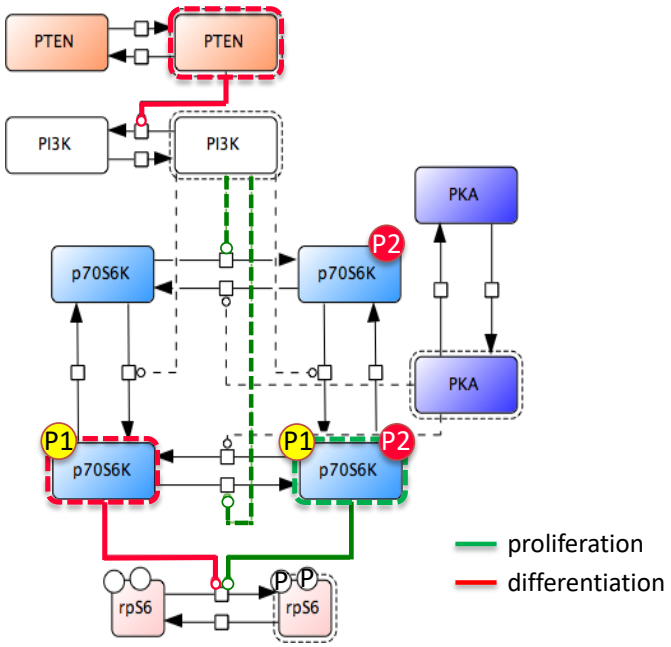


Figure 3: FSH-induced switch in Sertoli cells

Top panel: Sertoli cells undergo a sequence of morphological changes, leading them from round, undifferentiated cells at birth, to highly polarized and specialized cells in adulthood able to support spermatogenesis (germ cells are reprensented in green on the lateral borders of Sertoli cells).

Bottom panel: The FSH-induced switch involves numerous biochemical steps at the transcription, traduction and post-traduction levels (miRNA control of mRNA stability). Translational effects are mediated in part through the phosphorylation of p70S6K (a key step in the assembly of the translation preinitiation complex) which is under the antagonistic control of the cAMP/PKA and PI3K/PIP3 pathways. Dashed lines correspond to indirect links, while solid lines correspond to direct biochemical reactions, in proliferating (green) and differentiated (red) Sertoli cells. Relations common to both cell stages are figured in black (Musnier et al., 2009). The pro-mitogenic effect of active p70S6K is counteracted by PTEN, which is required for Sertoli cells to achieve terminal differentiation (Dupont et al., 2010). Active forms of PKA, PTEN and rpS6 are represented by boxes surrounded with dashed lines.

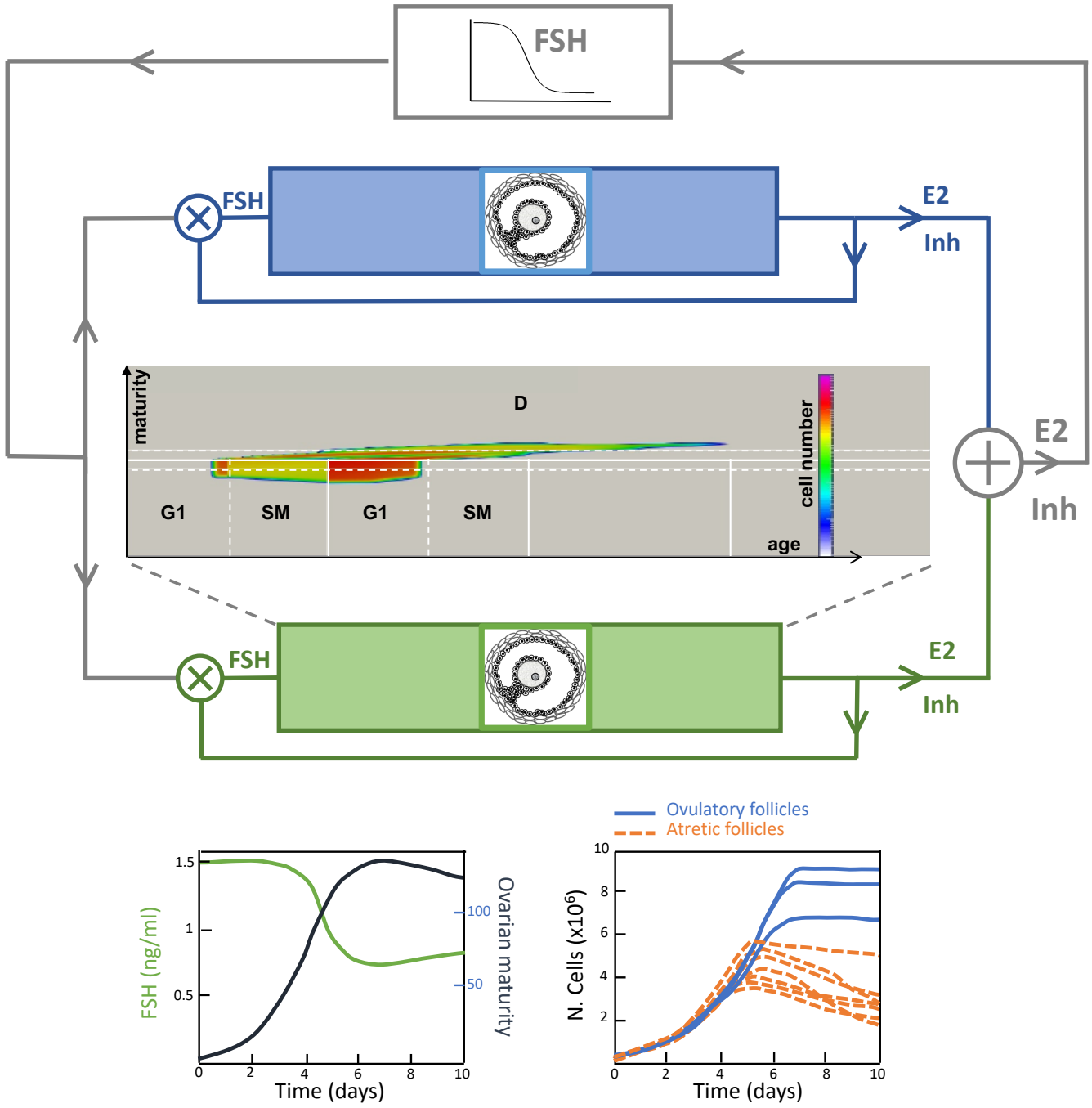


Figure 4: Multiscale modeling of the selection of ovarian follicles

Top panel: Microscopic outputs of the model and hormonal coupling between follicles. The cell dynamics within each follicle is ruled by population dynamics equations monitoring proliferation, differentiation and apoptosis rates according to the cell maturity and cytologic age. These dynamics are illustrated in the gray rectangle box, where the color code indicates the local cell density. Proliferation corresponds to the bottom part, with successive cell cycles (vertical solid white lines) divided into phase G1 and the remaining S-G2-M phases (vertical dashed white lines). Differentiation after cell cycle exit (horizontal solid white line) corresponds to the top part. Most apoptosis occurs during the switch from proliferation to differentiation (horizontal dashed white lines). Each follicle contributes to estradiol and inhibin secretion from the ovaries as a function of its global maturity (roughly speaking the product of the total cell number by the average cell maturity). The ovarian feedback onto the pituitary gland tunes the FSH levels. FSH availability is modulated locally, on the follicle level. FSH controls in turn the follicle cell dynamics, closing the loop and coupling the follicles together. For the sake of readability, only two interacting follicles are drawn.

Bottom panels: Macroscopic outputs of the model in the instance of a 10-follicle cohort. Bottom left panel: decrease in FSH level as a function of the hormonal feedback exerted by the whole follicle population (secretion of inhibin and estradiol). Bottom right panel: simultaneous changes in the cell numbers of 10 growing follicles. In this specific instance, 3 out of 10 follicles (blue solid lines) reach a critical cell mass compatible with ovulation, while the others (orange dashed lines) degenerate through atresia.

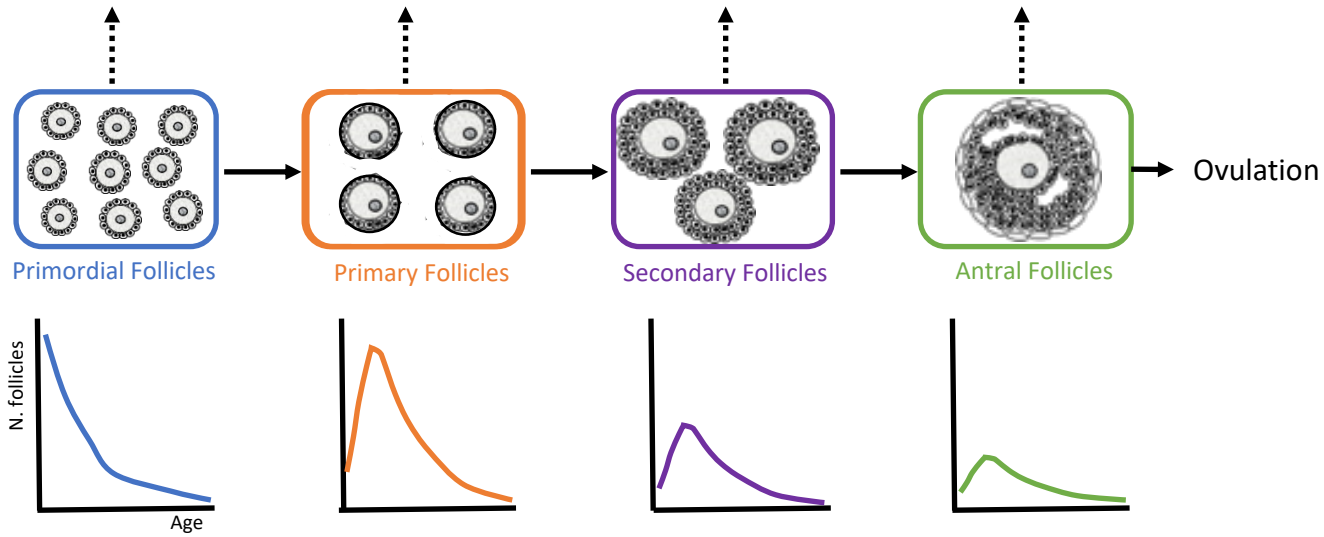


Figure 5: Modeling of the whole follicle development all along reproductive lifespan
The different stages of follicle development are represented by rectangle boxes. These stages are defined from morphological criteria such as the follicle diameter, granulosa cell number and presence of an antrum, and functional criteria such as estradiol production. An illustrative drawing of primordial, primary, secondary and antral follicles is shown in each box, from left to right. The model outputs (schematic graphs under each box) represent the changes in the follicle numbers with age. They are computed from the fitting of the growth rates (transfer from one box to the next, horizontal arrows) and atresia rates (follicle death, dotted vertical arrows) on experimental follicle counts.